EXAMPLE 1

Solid Phase Peptide Synthesis

Solid phase peptide synthesis (SPPS) was carried out on a 0.25 millimole (mmole) scale using an Applied Biosystems Model 431A Peptide Synthesizer and using 9-fluorenylmethyloxycarbonyl (Fmoc) amino-terminus protection, coupling with dicyclohexylcarbodiimide/hydroxybenzotriazole or 2-(1H-benzo-triazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/ hydroxybenzotriazole (HBTU/HOBT), and using *p*-hydroxymethylphenoxy-methylpolystyrene (HMP) or Sasrin™ resin for carboxyl-terminus acids or Rink amide resin for carboxyl-terminus amides.

Homocysteine (Hcy) was prepared by alkaline hydrolysis of L-homocysteine lactone or by reduction of homocystine using metallic sodium in liquid ammonia. Fmoc.Hcy(*S*-trityl) and Fmoc.Pen(*S*-trityl) were prepared from the appropriate precursor amino acids by tritylation with triphenylmethanol in trifluoroacetic acid, followed by Fmoc derivitization as described by Atherton *et al.* (1989, Solid Phase Peptide Synthesis, IRL Press: Oxford). 4-piperidinyl butyl ether derivatives of tyrosine (Y[$(CH_2)_4$-piperidine]) were prepared by SPPS starting with Fmoc-tyrosine-(4-Boc-piperidine butyl ether). Fmoc-*S*-(3-Boc-aminopropyl)cysteine was prepared from L-cysteine and Boc-aminopropyl bromide in methanolic sodium methoxide followed by treatment with O-9-fluorenylmethyl-O'-*N*-succcinimidyl carbonate (FmocOSu) at pH 10. 4-amidinophenylalanine (Amp) was prepared as described in co-owned and co-pending PCT International Patent Application Serial No. PCT/US94/03878, incorporated by reference.

Where appropriate, 2-haloacetyl groups were introduced either by using the appropriate 2-haloacetic acid as the last residue to be coupled during SPPS or by treating the N-terminal free amino group of the peptide bound to the resin with either 2-haloacetic acid/ diisopropylcarbodiimide/ *N*-hydroxysuccinimide in NMP or 2-halo-acetic anhydride/ diisopropylethylamine in NMP.

Where appropriate, 2-haloacetylated peptides were cyclized by stirring an 0.1 - 1.0 mg/mL solution in phosphate or bicarbonate buffer or dilute ammonium hydroxide (pH 8) containing 0.5 - 1.0 mM EDTA for 4 - 48 hours, followed by acidification with acetic acid,

ALLEGRETTI & WITCOFF, LTD.
10 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 715-1000